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(54) Title: FIBRIN-BASED SYSTEMS FOR THE CONTROLLED RELEASE OF MEDICINALS

(57) Abstract

A fibrin-based bio-erodible matrix for the controlled release of medicinals including protein therapeutics is disclosed. A method for controlled drug release is also disclosed.

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FIBRIN BASED SYSTEMS FOR THE CONTROLLED RELEASE OF MEDICINALS

FIELD OF THE INVENTION

This invention relates generally to a fibrin based bio-erodible delivery system which enables timed release of medicinals including proteins and small molecules. Fibrinogen is converted to fibrin in the presence of the medicinal which is entrapped when the fibrin gels.

BACKGROUND OF THE INVENTION

The rapid progress in recombinant DNA technology has provided researchers and clinicians with a variety of newly discovered proteins in amounts sufficient to enable laboratory and clinical study (Cytokines, A. Meager, Prentis Hall, 1991). Proteins either currently being administered by physicians or under investigation include growth factors, interferons, colony stimulating factors, and interleukins. In nature, these molecules may act locally as paracrine agents; i.e., they interact with and activate nearby cells. Further, they can be pleiotropic, i.e., they can activate or stimulate more than one kind of cell.

Delivery of these highly potent molecules for treatment of disease remains a challenge. Serious toxicity, low maximum tolerated doses (MTD), and limited therapeutic windows have been observed when given parenterally. As noted above, since some of these molecules are paracrine agents, localized delivery is another issue (Golumbek, P.T., et al, Cancer Research, 53, 5341 (1993)). As an example, systemic use of certain colony stimulating factors may result in autoimmunity and tissue damage from intense inflammatory reactions. Temporary relief of illness may be followed by permanent damage to the immune system.

Many novel proteins now being investigated for clinical use have very short half-lives. Clearance from the circulation can occur in a few hours or even a few minutes. Hence, prolonged release of effective doses below toxic levels would be advantageous.

Recombinant hormones such as bovine growth hormone (BGH) are widely used in dairy cattle. BGH is currently administered biweekly by injection. Controlled release of protein components in veterinary vaccines is desirable. Reduction of the frequency of injection and improvement in performance of the bio-active protein would be advantageous.

Bio-erodible polymers have been used for encapsulation of numerous classes of drugs (U.S. Patent No. 4,349,530; Royer, G.P., et al, J. Parenteral Science & Technol., 37, 34 (1983); Lee, T.K., et al, Science, 213, 233 (1981); WO91/06287 (1991); WO93/25221 (1993), all of which are hereby incorporated by reference). Synthetic polymers and copolymers of lactic acid and glycolic acid have been extensively investigated (U.S. Patent 5,122,367; Langer, Science, 249, 1927 (1990); U.S. Patent 4,983,393 (1991)). Autologous albumin and gelatin are also exemplified in the literature (U.S. Patent No. 4,349,530; Royer, G.P., et al, J. Parenteral Science & Technol., 37, 34 (1983); Lee, T.K., et al, Science, 213, 233 (1981)). Cross-linking with glutaraldehyde is known to stabilize albumin and gelatin matrices. Glutaraldehyde, however, is non-specific in its reaction with proteins. In solution glutaraldehyde forms a polymer which contains Michael addition sites. Functional groups such as amines and thiols react at these sites to form stable addition products. As a result, the protein drug can be inactivated or covalently bound to the matrix components. The latter reaction lowers the effective amount of deliverable drug or creates an antigenic molecule. A negative consequence of the latter chemical reaction is the development of autoimmunity.

U.S. Patent 4,983,393 discloses a composition for use as an intra-vaginal insert comprising agarose, agar, saline solution glycosaminoglycans, collagen, fibrin and an enzyme.

U.S. Patent 3,089,815 discloses an injectable pharmaceutical preparation composed of fibrinogen and thrombin. No provisions are made for protection of the medicinals from the action of thrombin.

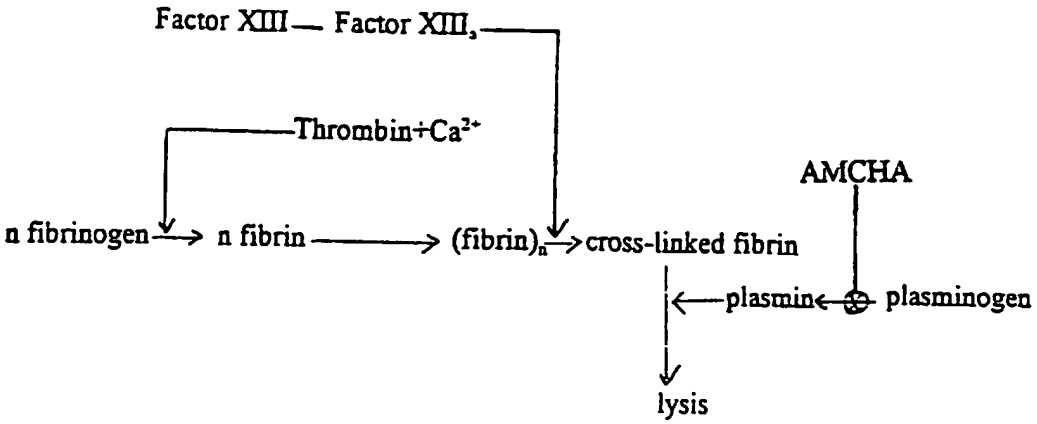
PCT Application WO/92/09301 discloses fibrin glue that is supplemented with at least one growth factor. U.S. Patents, 4,377,572 and 4,627,879 describe the use of fibrin in externally applied tissue sealant and topical wound treatments.

Many bio-erodible carriers are hydrophobic in nature and produce acid in conjunction with their hydrolysis. Examples include polymers of alpha hydroxy acids and various dicarboxylic acids. Although useful for delivery of small molecules and some polypeptides of low molecular weight, PLGA (co-polymer of lactic acid and glycolic acid), for example, is not generally useful for proteins (Langer, R., Science **249**, 1527 (1990).

Matrices stabilized by covalent cross-links (Lee, T. et al, Science **213** 233 (1981); Royer, US Patent 4,349,530; Golumbeck, P.K., Cancer Research 53, 5841 (1993), suffer since the medicinal protein can be inactivated or coupled to the matrix as a result of reaction with the cross-linking reagent. Moreover, harmful immunogens can be generated by these reactions.

In the final steps of blood clotting, fibrinogen is converted to fibrin which aggregates to form a strong gel. When factor XIII is present the gel is further stabilized by a specific enzyme-catalyzed transpeptidation reaction which covalently links fibrin segments.

Formation of a fibrin matrix from fibrinogen



The enzyme thrombin catalyzes the conversion of fibrinogen to fibrin; it also activates Factor XIII to become Factor XIII$_a$. The primary function of thrombin is to convert fibrinogen to fibrin. Similar to most proteases, thrombin will catalyze the rapid hydrolysis of small molecules and proteins, other than its natural substrate, examples include:

Thrombin Susceptible Bonds	Proteins
Arg-Asp	Secretin
Arg-Val	Cholecystokin
Arg-Cys, Arg-Asp, Arg-Gly	human chorionic gonadotrophin
Arg-Gly	Insulin B-Chain

Pre-formed fibrin has been used as an excipient for formation of implants in combination with various inorganic chemicals (Brown, R.G., et al., US Patent 4,393,041). These implants have been used for delivering steroids in farm animals. Fibrin is isolated, dried at high temperature and powdered to form the starting material. A drug, of low molecular weight, and other components are mixed prior to pelletization. Similar pre-formed fibrin preparations of bovine origin have been used

(again in combination with other materials) for formation of human prosthetic devices (Capperauld, R., et al., Surgery, Gyn. and Ob. 144, 3 (1977)).

Plasmin results from the activation of the precursor protein plasminogen. This process is inhibited by low molecular weight compounds such as amino caproic acid and trans-4-(aminomethyl) cyclohexanecarboxylic acid (AMCHA). The latter compound is more potent than amino caproic acid and is the active ingredient in an FDA approved hemostatic having the trade name "Cyklokapron."

Failures of conventional delivery systems for proteins are typically attributable to lack of design for controlled release, denaturation/inactivation of the medicinal protein in the matrix, adverse immunological reactions, or chemical modification of the medicinal during formulation.

OBJECTS OF THE INVENTION

It is an object of the invention to provide a bio-erodible delivery system which enables timed release of medicinals.

It is an object of the invention to provide a delivery system for proteins which does not alter the biological activity of the proteins.

It is a further object of the invention to provide a delivery system where the release profile is easily altered.

SUMMARY OF THE INVENTION

The subject invention relates to a medicinal delivery system based on the enzyme-catalyzed conversion of fibrinogen to fibrin, which forms a gel thus entrapping the medicinal. For certain protein medicinals, after adequate fibrinogen conversion but prior to gelation, a thrombin inhibitor is added along with the medicinal protein to protect the medicinal protein from fragmentation by the action of thrombin. The erosion rate of the matrix is altered by incorporation of fibrinolysis inhibitors, adjusting the concentration of matrix components, and including Factor XIII at various levels.

The invention also includes methods for obtaining sustained release of a medicinal and treating pain comprising administering the delivery systems of the invention to a mammal.

The invention includes methods of synthesizing a drug delivery system comprising the steps of mixing fibrinogen, thrombin, medicinal, and other optional components such as inhibitors of fibrinolysis, Factor XIII, albumin and collagen, and shaping the mixture.

Additionally, the invention includes kits comprising the components needed to make the delivery system.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows an idealized release profile of the subject invention.

FIGURE 2 shows a release profile of azo-albumin from a disc of fibrin matrix (13mm x 3mm).

DETAILED DESCRIPTION OF THE INVENTION

The attributes of a delivery system for medicinal proteins include:

-mild, non-denaturing preparative conditions
-hospitable environment--hydrophilic, neutral
-bio-erodible
-non-immunogenic, non-pyrogenic
-stable, sterilizable
-programmable, i.e., the release profile is adjustable by varying preparative conditions, components, and component concentrations.

The present invention relates to a fibrin based delivery system for the controlled release of medicinals including proteins and small molecules. Thrombin catalyzed polymerization of fibrinogen can be employed to entrap medicinal proteins to produce bio-erodible dosage forms in a variety of shapes. Absence of chemical cross-linking agents prevents potentially harmful modification of the medicinal protein or non-protein drug.

Fibrinogen is available commercially or it can be isolated from plasma, for example, by alcohol fractionation. Cryoprecipitates containing fibrinogen are alternatively used.

The enzyme thrombin is also commercially available. Alternatively, some fractions of fibrinogen contain sufficient thrombin to catalyze the gelation reaction in the presence of added $CaCl_2$.

Thrombin inhibitors such as antithrombin III, hirudin and hirudin fragments (see 1995 Sigma Catalog) are commercially available. These inhibitors are useful when the medicinal protein can be cleaved (and rendered inactive) by thrombin.

Calcium is available in the form of $CaCl_2$ solution, $CaCl_2$ finely ground, poorly soluble calcium compounds such as encapsulated calcium $CaCO_3$, and calcium propionate. Calcium ion can be withheld until after the dosage form is shaped. For example, after dispersion of the mixture (Ca-free) into droplets, finely ground Ca salts or solutions of Ca^{2+} can be added to bring about gelation.

Biocompatible additives such as albumin, collagen, globulin, polyvinylpyrrolidone, polyethyleneglycol, polylysine, etc. are optionally added to alter the release profile of the delivery system.

Fibrinolysis, catalyzed by the enzyme plasmin, is slowed by Factor XIII-induced stabilization. Therefore, including Factor XIII will reduce the erosion rate and extend the lifetime of the dosage form.

The erosion rate is reduced and the lifetime of the dosage form is extended by incorporation of fibrinolysis inhibitors such as alpha-2-antiplasmin, aprotinin, and AMCHA.

Production of the Delivery System

It is advantageous to use a two step process consisting of a formation step followed by a gelation step. Examples of ratios of components are as follows:

fibrinogen (10-90 mg/ml)

medicinal protein (.01-15 mg/ml)/thrombin inhibitor (0.01-10 unit/ml)

thrombin (.01-10 unit/ml)

calcium (.1-1M)

All of the above components except the medicinal protein are mixed and allowed to react at room temperature. Prior to gelation, the medicinal protein and the optional thrombin inhibitor are added and the mixture dispersed with stirring into a

water-immiscible medium such as mineral oil or vegetable oil. After the microspheres have formed, the temperature is optionally raised, for example, to 37°C. The temperature should not be high enough to denature the protein.

Further stabilization occurs in the presence of Factor XIII (typically, .1-1μM is used). Optionally, an amine-acceptor is used with the Factor XIII such as a lysine containing peptide (e.g., polylysine). A fibrinolysis inhibitor is optionally added.

The delivery system can be formed as beads, granules, microspheres (100-200 microns), threads, cylinders, disks, films or cell-sized microspheres (less than 100 microns) using techniques presented herein and known to those skilled in the art. Microbeads can be formed by dispersing a mixture of components in a water-immiscible medium with stirring. Subsequently, bead stabilization is accelerated by increasing the temperature to 37°C. Other geometric forms are produced using a similar two-step process. Adjustments of component concentrations and order of addition also permit direct injection of a reaction mixture which solidifies at 37°C in the body.

Implantable discs can be made, for example, by mixing all components at room temperature or below, pouring the mixture into a cylindrical mold and then curing at 37°C. The extruded fibrin cylinder is then sliced into discs.

Sub-batches of beads (or other shapes) can be prepared with relatively low levels of Factor XIII or an inhibitor of fibrinolysis. These sub-batches can be used to constitute blends of beads. To illustrate, when these blends of beads contain proportionately more of Factor XIII, the release is relatively slow. Blends of beads weighted toward low levels of Factor XIII release drug relatively quickly in proportion to the faster erosion rate.

By adjusting proportions of thrombin and fibrinogen, see e.g., Example 3, direct injection of a liquid is possible. In this embodiment, fibrinogen, thrombin, calcium chloride, along with optional additives such as Factor XIII, a fibrinolysis inhibitor, albumin, etc., are mixed and allowed to react. Prior to gelation, the medicinal protein and the optional thrombin inhibitor are added. The mixture is injected, s.c., after which the formulation becomes a solid at body temperature.

Fibrinolysis inhibitors are optionally added to extend the residence time of the formulation. Inhibitors can be included in microbeads which are included within the formulation or which are delivered separately. These "guardian" beads release inhibitor at a rate which controls erosion of the fibrin matrix. Simultaneous use of free and bead-entrapped fibrinolysis inhibitor such as AMCHA can be used advantageously to control fibrinolysis and therefore the residence time. For delivery of small molecules, this approach can be employed to preserve the fibrin depot for a period sufficiently long to permit release of most of the drug. AMCHA can be entrapped in PLGA (50:50) microspheres which are small enough to be injected using commonly used procedures and instruments.

Fibrin dosage forms of the subject invention exhibit residence times in vivo of up to three weeks or more.

Medicinal Proteins

As used herein, the term "medicinal" includes proteins as well as small molecule agents. The term "protein" includes naturally occurring proteins, recombinant proteins, protein derivatives and polypeptides. Medicinal proteins useful in the subject invention include colony stimulating factors (CSF) including G-CSF, GM-CSF, and M-CSF; erythropoietin (EPO); interleukins, IL-2, IL-4, IL-6, etc; interferons; growth factors (GF) including epidermal-GF, nerve-GF; tumor necrosis factor (TNF); hormones/bioactive peptides; ACTH; angiotensin, atrial natriuretic

peptides, bradykynin, dynorphins/endorphins/β-lipotropin fragments, enkephalin; gastrointestinal peptides including gastrin and glucacon; growth hormone and growth hormone releasing factors; luteinizing hormone and releasing hormone; melanocyte stimulating hormone; neurotensin; opiode peptides; oxytocin, vasopressin and vasotocin; somatostatin; substance P; clotting factors such as Factor VIII, enzymes used for "replacement therapy," e.g., glucocerebrosidase, hexoseaminidase A; and antigens used in preventative (such as tetanus toxoid and diphtheria toxoid), and therapeutic vaccines.

The matrices of the invention are very suitable for the continuous release of therapeutically active proteins and peptides (see table below) over extended periods of time, i.e., 1-6 days, 1, 2, 3 or 4 weeks or 1-2 months. The extent of the systemic (parenteral) treatment indications in humans is reflected by the examples in the following table.

Human Parenteral Treatment Indications: Proteins and Peptides

Medicinal	Clinical Indication
Hematopoietic growth factor G-CSF, GM-CSF, M-CSF, IL-3	bone marrow failure. Also immuno adjuvant, adjunct to myelosuppressive chemotherapy
erythropoietin	bone marrow failure; chronic anemia, kidney disease
adenosine deaminase, nucleoside phosphorylase	heritable immunodeficiencies
enzymes such as HGRPT, hypoxanthine-guanine phosphoriboxyl transferase	inborn errors of metabolism such as Lesch-Nyhan syndrome
enzymes such as ceredase	heritable lysosomal diseases such as Gaucher disease

enzymes such as arylsulfatases	heritable mucopolysaccharidoses such as Hurlers
pituitary hormones, such as growth hormone, gonadotrophins, vasopressin, et al	pituitary failure syndromes (hypothalamic pituitary disorders)
pituitary hormone analogs, such as analogs of LH-RH See e.g., Sigma Catalog #s L0637, L800L, L8886	disorders such as prostate hyperplasia and cancer, breast cancer, endometriosis
insulin	diabetes
cytokines such as TNF, or GM-CSF, interleukins such as IL-1, IL-2, IL-12	immunoadjuvant in vaccine administration such as hepatitis, herpes viruses, parasitic diseases, and cancer
interferons (alpha, beta and gamma)	hairy cell leukemia, viral infections
blood clotting factors such as factor VIII	bleeding disorders such as hemophilia
various immunoglobulins, hyper immune sera	therapy or prophylaxis in specific disease exposure or immunodeficiency state
vaccines such as hepatitis vaccines, herpes vaccines, antimicrobial vaccines, cancer vaccines	prevention of bacterial viral and parasitic diseases, cancers such as melanoma

In another embodiment of the invention, the medicinal protein is protected as described in commonly owned U.S. Serial No. 08/571,613 hereby incorporated by reference in its entirety. Medicinal proteins can be protected from proteolysis or other reactions by chemical modification of sidechains adjacent to susceptible bonds or which constitute reactive functional groups. In addition to chemical protection, the medicinal protein can be physically protected, that is, it can be employed as a crystal or solid matrix such as gelatin micro-beads. Such small particles can be dispersed into the fibrin liquid prior to gelation and then trapped within the depot. Any matrix

which does not react with medicinal can be employed as long as it is safe, bio-erodible, and does not interfere with the release.

In another embodiment of the invention, microbeads of the subject invention containing medicinal are encapsulated in the delivery system described in commonly owned U.S. Ser. No. 08/571,613, for example microbeads of the subject invention in a delivery system comprising albumen and/or collagen crosslinked with oxidized dextran.

There are many analogous parenteral and local therapies that are enhanced by this invention in veterinary medicine, especially when biologically active proteins and polypeptides are used, e.g., bovine and porcine growth hormone, tetanus toxoid, C. perfringens antigens (B,C,D), feline leukemia antigens and leptospirosis antigens.

Non-Protein Delivery

The present delivery system is also applicable to formulations with non-protein medicinals, including but not limited to alkaloids, steroids, terpenoids, amino acid derivatives, nucleoside/nucleotide derivatives, polynucleotides, carbohydrates, polysaccharides, lipids, lipopolysaccharides, purines, pyrimidines and derivatives of same.

Advantageous small molecule drugs include: analgesics, anesthetics (e.g., lidocaine or pramocaine), antialcohol preparations, anti-infectives, antiseptics (e.g., compounds of monovalent silver, silver benzoate, PVP-I_2), anticoagulants, anticancer drugs, antidepressants, antidiabetic agents, antihypertensive drugs, antiinflammatory agents (e.g., hydrocortisone), antinauseants, anorexics, antiulcer drugs, cardiovascular drugs, contraceptives, decongestants, diuretics, hormones/antihormones, immunosuppressives, narcotic detoxification agents, uricosuric agents, and wound healing promoters such as deoxyribonucleosides (see table below).

The delivery systems of the invention are also suitable for the prolonged, controlled release of non-proteinaceous compounds with therapeutic activity. For example, a matrix can be constructed that will enable the steady release of a local anesthetic such as lidocaine over several weeks such that an adequate concentration can be maintained for 1-6 days, 1, 2, 3 or 4 weeks or 1-2 months, in the immediate vicinity of nerve roots damaged by a pathologic vertebral fracture due to cancer thus relieving severe pain without causing irreparable loss of function.

Especially useful for post-surgical implantation are fibrin discs containing local anesthetic. The discs (e.g., 0.8cm in dia. x .3cm) release anesthetic (e.g., lidocaine or marcaine) over a period of at least three days. For example, following arthroscopic surgery of the knee, one disc is implanted laterally and one disc medially. This approach obviates systemic use of narcotic pain killers.

The extent and wide variety of uses of the delivery systems of the invention in conjunction non-proteinaceous molecules can be appreciated from the examples given in the following table.

Human Parenteral Treatment Indications: Non-proteinaceous Molecules

Medicinal	Clinical Indication
antibiotics such as penicillins, beta-lactams, amino-glycosides, macrolides, tetracyclines, etc.	bacterial infections, e.g., bone and joint infections, soft tissue infections in areas not sufficiently vascularized, Streptococcal sore throat or E. Coli urinary tract infection
Antifungals such as nystatin	thrush and other localized fungal infections
antiinflammatories such as hydrocortisone	inflammation due to arthritis, etc.
hormones such as testosterone, estrogens, adrenal steroids	contraception, various testicular, ovarian, adrenal failure states

immunosuppressants such as cyclosporin	organ/tissue heterotransplantation
anticoagulants such as heparin, coumadin	pulmonary embolus, vascular diseases
diuretics such as furosemide	cardiac failure
vermifuge such as mebendazole	various helminth parasitism
antimalarials such as chloroquine	treatment and prophylaxis of malarial parasitism
anesthetics such as lidocaine, marcaine or pramocaine	cancer pain, arthritis pain, post surgical pain

Analogous uses of small molecules in veterinary medicine are also included in this invention.

* * *

Formulations made possible by this invention enable the local therapeutic application of medicinals. For example, infected wounds or chronically infected ulcers such as those seen in patients with diabetes or vascular insufficiency to the lower extremities benefit from preformed films based on this invention that release debriding enzymes in combination with antibiotics and growth factors such as TGF-β or PDGF, to promote healing. The healing of clean surgical incisions is enhanced by the local application of beads or implants that release growth factors and optionally anti-infectives or a local anesthetic to relieve patient pain.

Particularly advantageous compounds for use in the subject invention are those in crystalline form.

Preformed films for transdermal delivery or for topical application as bandages can also be used. In this case the film may be used to deliver non-proteinaceous drugs such as anti-infectives and wound healing promoters.

* * *

Release of Medicinals and Modes of Administration

An idealized release profile is shown in Figure 1. Here the concentration of medicinal in the vicinity of the bead reflects the rate of internal degradation of the three different classes of gel matrices. The profile shown depicts the system with identical medicinal concentration in all classes. For example, to have higher levels of medicinal released at a later period, more medicinal would be incorporated in the Class III beads as shown in this example. Release profiles can be obtained from zero order release to those involving late-stage bursts. It is also possible to administer more than one medicinal in the same treatment regimen. The drugs can be released simultaneously or sequentially.

While not wishing to be bound by a particular theory, the rate of diffusion of a medicinal from a matrix to the therapeutic compartment is given by equation (1)

$$\text{rate} = DA(d[m]/dx) \qquad (1)$$

D is the diffusion coefficient

A is the surface area

d[m]/dx is the medicinal gradient

Stokes law is also applicable (2)

$$D = 1/rv \qquad (2)$$

r is the radius of the medicinal

v is the viscosity of the medium.

The diffusion coefficient (D) is inversely related to the viscosity of the medium (v), the size of medicinal (r), and also the erosion rate of the device. The matrix density and degree of cross-linking influence the viscosity. As the fibrin matrix is broken down, v is reduced which increases D which in turn increases the rate of diffusion. In the extreme case involving a dense matrix and a large medicinal molecule, the rate will be very low or zero until the breakdown occurs.

Another means of control of the release profile is the geometry of the delivery device. The surface area of sphere for example is given by (3)

$$A = 4\pi r^2 \qquad (3)$$

The volume of a sphere is given by (4)

$$V = 4/3\pi r^3 \qquad (4)$$

Combining (3) and (4) gives

$$A/V = 1/r \qquad (5)$$

As shown in equation (5), a bead of a given volume will have less surface area than multiple beads with the same aggregate volume. A blend of beads with a range of radii will tend to give a release profile resembling zero order.

Computer modeling of this system with adjustable parameters can be used to generate a set of hypothetical release profiles for a given therapeutic protein.

Solid Dosage Forms

Medicinal matrix of the invention is administered to a human or other mammal as beads, disks, threads and implants of various other shapes using techniques known to those skilled in the art. Beads would be normally administered via needle subcutaneously, intramuscularly, intraperitoneally, or intravenously for cell-sized microbeads. Tablets and capsules are used for oral delivery.

Injectable Liquid

In addition to implants and injectable beads, a liquid formulation can be directly injected subcutaneously, intramuscularly and intraperitoneally, which solidifies soon after injection. The liquid formulations are made such that the mixture remains a liquid until after injection. See Example 3.

Preformed Films

Films can be cast using the preparations described herein, for example the preparation of Example 4. One approach is to use a multi-channel pipette and apply the preparation to a glass plate. The texture of the resulting material varies depending on the humidity and temperature. Alternatively, the preparation is applied directly to the wound or to a bandage which is in turn placed on the wound. Medicinals for topical preparations include, for example, anesthetics (lidocaine, pramocaine), antiseptics (PVP-I_2, compounds of monovalent silver, polymyxin B, neomycin, gramicidin) and living cells. For abrasions and lacerations, the preparations of the invention provide beneficial osmotic draw as well as protection, pain relief and infection control. For burn treatment other agents such as growth factors or cultured cells are optionally included.

Medicinal matrix can be administered concomitant with surgical procedures. Examples of this include 1) an antibiotic matrix following abdominal surgery, 2) matrix containing cytotoxic chemotherapeutic drug following tumor removal, and 3) matrices containing adjuvants/antigens following tumor removal. Also, implants can be placed under the skin adjacent to the joint capsule, or elsewhere following orthopedic surgery.

* * *

The following Examples are illustrative, but not limiting of the compositions and methods of the present invention. Other suitable modifications and adaptations of a variety of conditions and parameters normally encountered in clinical therapy which are obvious to those skilled in the art are within the spirit and scope of this invention.

EXAMPLES

Example 1

Production of Implantable Discs or Cylinders

Solution A (400 µl) was made containing fibrinogen (25 mg/ml) in buffer--Hepes, 30 mM, pH 7.2, 0.15 M in NaCl. Solution B (100 µl) was made containing the enzyme thrombin (about 5 units) and $CaCl_2$ (1.6 M) in the same buffer. Solution C (100µl) was made containing protein (azo-albumin)(2mg/ml), and antithrombin III/heparin in sufficient quantity to neutralize the thrombin. Solutions were equilibrated to temperature (20°C).

Solutions A and B were mixed and allowed to react approximately 2 minutes (or until the viscosity had noticeably increased prior to gelation). Solution C was then added. After mixing, the resulting combination was injected into the mold.

Cylindrical molds were made by cutting off the needle ends of disposable syringes. The modified syringe was clamped in a vertical position and filled with the mixture of solutions A and B which had cured for three hours at 37°C. Step-wise extrusion and slicing allowed production of implantable discs. Small syringes of narrow bore can be used in a similar fashion to produce cylinders.

Controlled release occurs over a three week period. See Figure 2.

Example 2

Microsphere Preparation

Microspheres were made using known methods with or without low concentrations of surfactants. About 500ml of the water-immiscible medium, such as vegetable oil, was charged into a round-bottom flask with baffled sides. Agitation was provided by an overhead mechanical stirrer. Solutions A, B, and C were mixed as in Example 1 using the same proportions with the total final volume of 2 ml. The mixture was injected into the stirred water-immiscible medium at room temperature. The temperature was then raised to 37°C and the stirring was continued for 3 hr. The microspheres were collected by centrifugation and then washed quickly with ether on a filter.

Example 3

Direct Injection

The thrombin concentration is adjusted to allow direct injection. Solutions A, B and C are mixed as previously described and the mixture is injected s.c. into a mammal.

Example 4

Films

The procedure of Example 1 is repeated using other geometric configurations including films containing antibiotics and wound healing promoters. The reaction mixture is poured onto a flat glass plate with borders to provide boundaries of the desired dimensions. The glass plate is warmed to 37°C and allowed to stand for 3-8 hours in a humidity controlled chamber.

Example 5

Fibrin Based Formulation of Lidocaine

Solution A (400µl) was made containing fibrinogen (25mg/ml) in buffer--Hepes, 30 mM, pH 7.2, 0.15 M in NaCl. Finely ground lidocaine (50 mg) was then suspended therein. Solution B (100µl) was made containing thrombin (5 units) and $CaCl_2$ (1.6 M) in the same buffer. Solutions A and B were equilibrated to 20°C and mixed. When using a matrix protectant, such as AMCHA, it is included in Solution A by direct dissolution or by suspension if in the form of PLGA microspheres. AMCHA should be at least 10 µg/ml in the immediate vicinity of the drug depot.

Example 6

Beads Containing Dispersed Solid Medicinal

The procedure of Example 1 was repeated but with 10-40% by weight of a crystalline or amorphous solid medicinal protein suspended in the reaction mixture.

Example 7

Release Profile of an Antibiotic

A. Matrix Preparation

Fibrin stock solution (1 ml/3%) was mixed with 50 mg of gentamicin sulfate. Thrombin solution (25 µl/2.5 units) was added and the mixture was dispensed in 200 µl aliquots to screw-cap vials. After incubation for 1 hr at 37° the vials were allowed to stand at room temperature overnight. The samples were stored at 0-4°.

B. Release of Gentamicin

The buffer (1 ml/PBS with 0.01% azide) was pipetted into four vials, two of which served as controls. The buffer was changed at 24-hr intervals. Gentamicin concentration was determined using a standard curve prepared with known amounts using the TNBS test (Habeeb, A.F.S. A. 1966 *Anal. Biochem.* 14, 328). A release profile is shown below:

Time(Days)	% Released
1	32
2	13
3	5
4	6
5	6

* * *

It will be readily apparent to those skilled in the art that numerous modifications and additions may be made to both the present invention, the disclosed device, and the related system without departing from the invention disclosed.

WHAT IS CLAIMED IS:

1. A gel medicinal delivery system comprising

(i) fibrin, and

(ii) a protein medicinal not cleavable by thrombin.

2. A gel medicinal delivery system comprising

(i) fibrin,

(ii) a protein medicinal, and

(iii) a thrombin inhibitor.

3. A gel medicinal delivery system comprising

(i) fibrin,

(ii) a medicinal, and

(iii) an inhibitor of fibrinolysis.

4. A gel medicinal delivery system comprising

(i) fibrin,

(ii) a medicinal, and

(iii) Factor XIII optionally with an amine acceptor.

5. A system as in Claim 4 wherein said amine acceptor is a lysine containing peptide.

6. A system as in Claim 1 further comprising a compound selected from the group consisting of albumin, collagen, polyvinyl-pyrrolidone, polyethylene glycol and polylysine.

7. A system as in Claim 1 encapsulated in a gel matrix comprising at least one matrix protein selected from the group consisting of gelatin and albumin, and a polymeric stabilizer and/or an external cross linker.

8. A system as in Claim 1 further comprising Factor XIII.

9. A system as in Claim 3 wherein said inhibitor of fibrinolysis is selected from the group consisting of alpha-2-antiplasmin, aprotinin, pancreatic trypsin inhibitor and AMCHA.

10. A system as in Claim 1 wherein said system is in the form of a bead.

11. A system as in Claim 1 wherein said system is in the form of a disc.

12. A system as in Claim 1 wherein said protein medicinal is a protected protein.

13. A system as in Claim 1 wherein said protein medicinal is encapsulated in non-covalently linked microbeads.

14. A system as in Claim 2 wherein said protein medicinal is encapsulated in non-covalently linked microbeads.

15. A system as in claim 13 wherein said microbeads are gelatin microbeads.

16. A system as in Claim 1 wherein said protein medicinal is in crystalline form.

17. A system as in Claim 3 further comprising a thrombin inhibitor.

18. A system as in Claim 2 wherein said thrombin inhibitor is antithrombin III/heparin.

19. A system as in Claim 1 wherein component (i) comprises 0-80% by weight fibrin and 0-80% by weight medicinal.

20. A sustained release delivery system comprising:

(a) a first gel matrix comprising:

(i) fibrin,

(ii) an inhibitor of fibrinolysis, and

(iii) a medicinal protein,

and

(b) a second gel matrix comprising:

(i) fibrin,

(ii) an inhibitor of fibrinolysis at a different level than is said first gel matrix, and,

(iii) said medicinal protein.

21. A blend of gel matrices for sustained release of a medicinal comprising at least two gel matrices according to Claim 3 wherein said gel matrices have different levels of inhibitor of fibrinolysis.

22. A blend of gel matrices for sustained release of a medicinal comprising at least two gel matrices according to Claim 4 wherein said at least two said gel matrices have different levels of Factor XIII.

23. A blend of gel matrices for sustained release of a medicinal comprising at least two gel matrices according to Claim 1 wherein said at least two said gel matrices have different levels of gel density.

24. A method for obtaining sustained release of a medicinal protein comprising administering the system of Claim 1 to a mammal.

25. A method for obtaining sustained release of a medicinal protein comprising administering the system of Claim 3 to a mammal.

26. A method for obtaining sustained release of a medicinal protein comprising administering the system of Claim 20 to a mammal.

27. A method of treating pain in a mammal in need of such treatment comprising administering the system of Claim 3 to said mammal wherein said medicinal is an anesthetic.

28. A method of treating pain in a mammal in need of such treatment comprising administering the system of Claim 4 to said mammal wherein said medicinal is an anesthetic.

29. A method as in Claim 24 wherein said administration is subcutaneously.

30. A method as in Claim 25 wherein said administration is subcutaneously.

31. A method as in Claim 25 wherein said inhibitor of fibrinolysis is administered separately.

32. A method as in Claim 25 wherein said inhibitor of fibrinolysis is in the form of microbeads.

33. A method of synthesizing a drug delivery system comprising the steps of:

a) mixing fibrin, medicinal protein, calcium ion to form a gel matrix,

b) shaping said gel matrix,

c) curing said gel matrix.

34. A method as in Claim 33 wherein said mixing step includes mixing thrombin.

35. A kit comprising:

(i) a vial containing fibrinogen,

(ii) a vial containing thrombin and calcium ion, and

(iii) a vial containing medicinal protein and a thrombin inhibitor.

36. A kit comprising:

(i) a vial containing fibrinogen,

(ii) a vial containing thrombin and calcium ion, and

(iii) a vial containing medicinal protein not cleaved by thrombin.

37. A kit comprising:

(i) a vial containing fibrinogen,

(ii) a vial containing thrombin and calcium ion, and

(iii) a vial containing medicinal protein and an inhibitor of fibrinolysis.

38. A kit as in Claim 35 further comprising a vial containing a Factor XIII.

Fig. 1

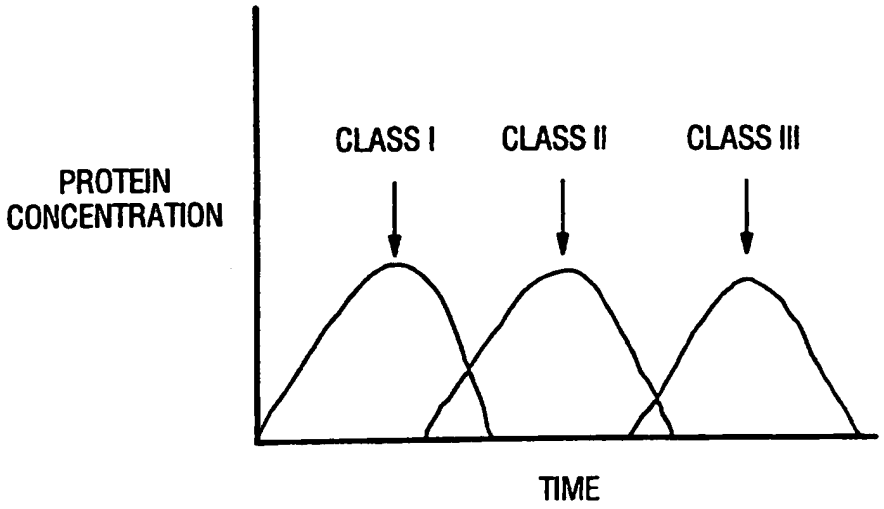
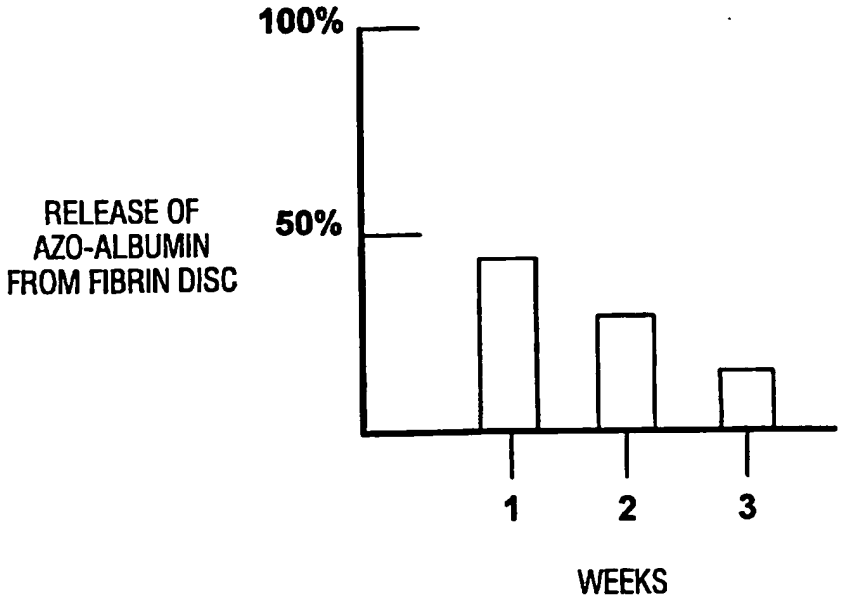

CLASS I
CLASS II
CLASS III
PROTEIN CONCENTRATION
TIME

Fig. 2


100%
50%
RELEASE OF AZO-ALBUMIN FROM FIBRIN DISC
1
2
3
WEEKS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/08909

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/48, 38/36
US CL : 424/94.64, 422, 443, 484, 486, 491, 499; 530/382; 514/21
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.64, 422, 443, 484, 486, 491, 499; 530/382; 514/21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	HO et al. Fibrin-based drug delivery systems. II. The preparation and characterization of microbeads. Drug Development and Industrial Pharmacy. 1994, Volume 20, Number 4, pages 535-546, particularly page 539.	1, 8, 10, 13, 18 ---------- 9
X ---- Y	HO et al. Fibrin-based drug delivery systems III: The evaluation of the release of macromolecules from microbeads. Journal of Controlled Release. 1995, Volume 34, pages 65-70, see abstract.	1, 5, 8, 10, 13, 18 ---------- 9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 SEPTEMBER 1997	01 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHHAYA SAYALA Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/08909

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 3,089,815 A (LIEB et al.) 14 May 1963, see column 1, column 2, lines 70-75, column 4, line 10, column 7, column 8, and column 12.	1, 10, 13, 18, 23, 27-30 --- 2, 5, 11-12, 22, 31-32
X --- Y	US 5,290,552 A (SIERRA et al.) 01 March 1994, see column 3, lines 1-10, column 4, and column 5.	1, 3-7, 10, 13 --- 8-9, 11-12, 14-32
Y	SENDEROFF et al. Fibrin Based Drug Delivery Systems. Journal of Parenteral Science and Technology. January-February 1991, Vol. 45, No. 1, pages 2-6, see entire document.	9
Y	BOYCE et al. Delivery and Activity of Antimicrobial Drugs Released from Human Fibrin Sealant. Journal of Burn Care Rehabilitation. May/June 1994, Volume 15, pages 251-255, see entire document.	9
X	US 4,393,041 A (BROWN et al.) 12 July 1983, see claims 1, 3-4 and 6.	1, 5, 10, 13 --- 8-9
X	US 5,171,579 A (RON et al.) 15 December 1992, see column 1, column 4, and claims 4-7.	1, 10, 18
Y	US 4,548,736 A (MULLER et al.) 22 October 1985, see whole document.	1-2, 4-6, 8-13, 16-18, 22-23, 27-30 and 32
X, E --- Y, E	US 5,643,596 A (PRUSS et al.) 01 July 1997, see whole document.	1, 3, 5, 7, 10, 13, 18-19 --- 8-9, 14-15, 17, 21-22, 24, 26 and 32
Y, P	US 5,631,011 A (WADSTROM) 20 May 1997, see whole document.	1-2, 4-6, 8-13, 16-18, 22-23, 27-20 and 32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/08909

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAPLUS, EMBASE, MEDLINE, BIOSIS, WPIDS.

search terms: fibrin, fibrinogen, carrier, implant, delivery, matrix, aprotinin, factor XIII, thrombin.